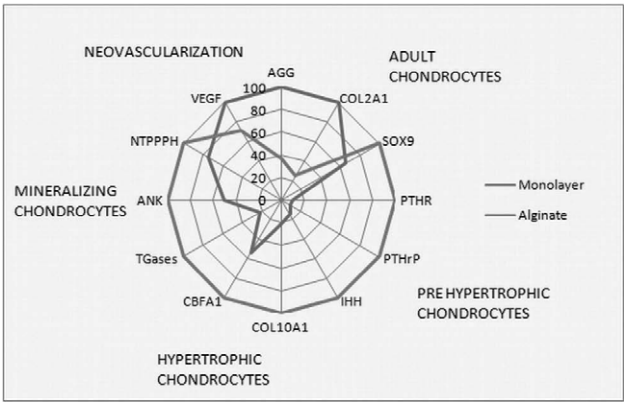

Figure 1. Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in 10% FBS media, n=3.

activity, transglutaminase activity and 5' phosphodiesterase activity of NTPPPH were quantified by specific enzymatic methods
Results: In 10% FBS, chondrocytes expressed significantly more hypertrophic (PTH-R, PTHrP, ihh, COL10A1 and cbfa1) and matrix mineralization genes (TG2, FXIIIA, CILP, ENPP1 and Ank) in alginate beads than in monolayer (Figure 1). Whatever the culture system (alginate beads or monolayer), chondrocytes cultured in 10% FBS expressed significantly more hypertrophic and matrix mineralization genes like COL10A1 than chondrocytes cultured in 1% ITS+ or 2% UG (Figure 2).

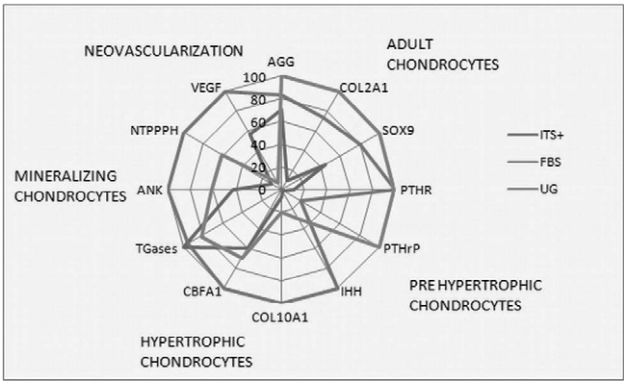

Figure 2. Expression of genes and enzymatic activities of chondrocytes after 21 days of culture in alginate beads, n=3.

Conclusions: The expression of hypertrophic markers in articular chondrocytes is upregulated in fetal bovine serum (FBS) and alginate beads culture conditions, suggesting that cartilage neovascularization could be a factor of chondrocyte hypertrophic differentiation in OA cartilage

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MOLECULAR COMPARISON OF PRIMARY HUMAN CHONDROCYTES, IMMORTALIZED HUMAN CHONDROCYTES (T/C-28A2 AND C-28/I2), AND A HUMAN CHONDROSARCOMA CELL-LINE (SW1353) USING GLOBAL TRANSCRIPTIONAL PROFILING

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Purpose: Primary human chondrocytes are difficult to obtain, and their gene expression and response to cytokine stimuli are often inconsistent from donor to donor since factors such as disease severity and donor age are complicating variables. Therefore, immortalized chondrocyte-based cell lines are tempting alternatives for use in cell-based assays. However, these cell lines are phenotypically different from the primary chondrocytes (more fibroblast-like, minimal extracellular matrix secretion) and a limited molecular characterization has revealed some differences (Finger et al., 2003), yet no extensive analysis has been performed previously.
Methods: In order to investigate in detail the similarities and differences between primary chondrocytes and the chondrocytic cell lines T/C-28a2, C-28/I2 and SW1353, cells were treated in monolayer culture with or without IL-1 (1ng/ml) or TNF (10ng/ml) for 18 hours and global transcriptional changes were studied using Affymetrix gene chips. Focused gene expression was also analyzed using a TaqMan Low Density Array (TLDA) Immune Panel from Applied Biosystems, which measures the transcriptional levels of 90 immune- and inflammation-related genes simultaneously.
Results: Global profiling using correlation maps and hierarchical clustering indicated that transcriptional patterns in T/C-28a2, C-28/I2 and SW1353 are significantly different than in the primary chondrocytes. The transcriptional patterns of these samples clustered more closely to fibroblastic cell lines, such as BJ cells, than to the primary chondrocytes. The magnitude of transcriptional response to cytokines by primary chondrocytes was generally more than 10 fold greater than that of the immortalized cell lines. Transcriptional profiles of the chondrocyte cell lines T/C-28a2 and C-28/I2 were also significantly different from each other, and genes that were differentially expressed in the two cell lines were identified. Clustering analysis, Welch t-tests, as well as comparison of selected chondrocyte-specific gene expression patterns showed that the T/C-28a2 cell line is more similar to the human primary chondrocytes than the C-28/I2 line. Cytokine responsive gene sets common to all of the cells as well as gene sets unique to each cell type were identified for both IL-1 and TNF. Finally, TLDA analysis of immune related gene expression allowed detection of gene expression that was not measurable by gene chip analysis due to its lower sensitivity.
Conclusions: Our transcriptional analysis study using gene chip and TLDA showed that the immortalized chondrocytes (and SW1353 chondrosarcoma cells) that are frequently used in cartilage research are significantly different from primary chondrocytes. These results should be taken into consideration before choosing these cells as a substitute for primary chondrocytes in *in vitro* experiments.

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CORM-2 DOWN-REGULATES DEGRADATIVE ENZYMES AND ENHANCES EXTRACELLULAR MATRIX COMPONENTS IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: The articular chondrocyte is responsible for both matrix production and destruction. Excessive collagen II degradation is accompanied by loss of aggrecan in osteoarthritis (OA). Collagen II breakdown is mediated primarily by collagenases while aggrecan degradation is dependent on the activation of matrix metalloproteinases (MMPs) and aggrecanases. We have recently shown that the CO-releasing molecule tricarbonyldichlororuthenium(II) (CORM-2) inhibits aggrecan degradation in OA cartilage explants. The objective of this work was to investigate the effects of CORM-2 on extracellular matrix components and the mechanisms involved.
Methods: Cartilage specimens were obtained from 15 patients with diagnosis of advanced OA undergoing total knee joint re-

placement. Chondrocytes were isolated by digestion with collagenase and used in primary culture. Cells were stimulated with IL-1β for different times in the presence or absence of CORM-2 (50, 100 or 150 μM). Gene expression was analyzed by real-time PCR. Protein expression was investigated by Western blot method and immunocytochemistry. Aggrecanase activity, MMP protein levels and phosphorylated/total levels of ERK and IκBα were determined by ELISA, and NF-κB activity by the luciferase method.
Results: CORM-2 treatment increased cell viability and collagen II and aggrecan protein expression in primary OA chondrocytes. Increased gene expression of collagen II, aggrecan, link protein, Sox-9 and BMP2 was observed. Interestingly, aggrecanase activity was reduced by CORM-2 in a concentration-dependent manner, as well as gene expression and protein levels of MMP-1, MMP-3 and MMP-10. Our results also indicate that down-regulation of cartilage degradative enzymes by CORM-2 may be mediated by the inhibition of ERK and IκBα phosphorylation, and of NF-κB activation.
Conclusions: CO released by CORM-2 enhances matrix components and decreases the production of MMP and aggrecanase activities induced by IL-1β in OA chondrocytes, indicating the potential interest of this class of compounds in cartilage protection.

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LEPTIN INDUCES DETRIMENTAL CHANGES IN HUMAN OA CARTILAGE. EFFECTS ON NITRIC OXIDE, PGE2, IL-6 AND IL-8 PRODUCTION

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Purpose: Obesity is an important risk factor for OA of weight-bearing joints, but also for hand joints pointing to an obesity-related metabolic factor that influences on the susceptility or pathogenesis of OA. Leptin is a proinflammatory adipokine regulating energy balance and it has lately been related also to arthritis and cartilage metabolism. In the present study, we investigated the effects of leptin on human OA cartilage.
Methods: Cartilage tissue obtained from the leftover pieces of total knee replacement surgery from patients with OA were used in the experiments.
Results: Leptin alone and in combination with IL-1 induced iNOS expression and NO production, COX-2 expression and PGE2 production, and IL-6 and IL-8 secretion in human OA cartilage in a concentration dependent manner. The effects of leptin were mediated through transcription factor NF-kB and MAP kinases JNK and p38 (the latter only in the case of COX-2 and PGE). In addition, JAK-STAT pathway seems to mediate the leptin effect on iNOS and NO. There were significant inter-individual differences in the responsiveness to exogenous leptin, and its relation to BMI, severity of OA, and SOCS-3 expression was assessed.
Conclusions: The findings support the idea of leptin as a detrimental factor in OA cartilage and as a link between obesity and increased risk for osteoarthritis.

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CHONDROCYTE HYPERTROPHY IN ANK-/ANK- MICE: IMPLICATIONS FOR OSTEOARTHRITIS

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Purpose: Chondrocyte hypertrophy is a morphologic feature of both the normal calcified cartilage layer of the articular plate and of uncalcified cartilage in pathologic states such as osteoarthritis. The relationships of chondrocyte hypertrophy to chondrocyte matrix domains and to cartilage maturation, although commonly observed, are not well understood. As we have recently demonstrated (Osteoarthritis and Cart, 14(Suppl B 2006): pS147, December 2006), $ank^{-/-}$ mice show an increased population of hypertrophic chondrocytes in the uncalcified cartilage layer. To determine whether chondrocyte hypertrophy is a generalized feature of $ank^{-/-}$ mice and to determine implications on calcified cartilage matrix and subchondral bone, we extended our studies to examine chondrocyte size in calcified and epiphyseal cartilages and their relationships to calcified cartilage and subchondral bone thickness.
Methods: Knee joints in 18 mice, homozygous ($ank^{-/-}$), and controls ($ank^{+/+}$), 3 mice each at each of the following 3 time points: 6 weeks, 12 weeks and 18 weeks were studied. Five μm sections from fixed, decalcified, paraffin-embedded knee joints were stained with hematoxylin and eosin. Chondrocyte size distribution and cartilage matrix domains were determined for uncalcified, calcified and epiphyseal cartilages, as well as subchondral bone thickness. The measurements were performed on a Visiopharm Integrator Image Analysis System, Leica DM 4500 3 microscope and an Olympus D70 camera. With selection of the central 50% of the tibial plateau articular plate and underlying epiphysis, as the region of interest, the chondron areas of chondrons containing nucleated chondrocytes were measured for uncalcified, calcified and epiphyseal cartilage. As well, uncalcified cartilage, calcified cartilage and subchondral bone thicknesses and areas were measured over the study region.
Results: We observed that compared to wild type controls, ank chondrocytes in uncalcified cartilage and epiphyseal cartilage at all ages were enlarged. In contrast, within calcified cartilage, the ank chondrocytes were smaller than controls at 12 and 18 weeks. Calcified cartilage in ank mice was significantly thicker at all ages. Conversely, subchondral bone thickness in ank mice although thicker than controls at 6 weeks, was significantly thinner at 18 weeks of age.

Results: typically at 18 weeks

	Wild Type	ank Mice
Calcified cartilage thickness (μm)	59±12	86±11
Subchondral bone thickness (μm)	423±18	331±12

Conclusions: ank-/ank- mice have a generalized defect in chondrocytes which leads to chondrocyte hypertrophy of uncalcified and epiphyseal chondrocytes. These changes are associated with increased calcified cartilage thickness, which in turn appears related to decreased bone thickness within the articular plate. The intriguing predicted implication is that uncalcified chondrocyte hypertrophy, which in osteoarthritis is focal, may result in focally thinner underlying subchondral bone. This subchondral bone, in turn, would more readily permit microfractures leading to articular plate remodelling, a characteristic feature of osteoarthritis.